Institut für Parasitologie
der Vetsuisse-Fakultät Universität Zürich
Direktor Prof. P. Deplazes

Arbeit unter der Leitung von Dr. P. Torgerson

Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models

Inaugural-Dissertation

zur Erlangung der Doktorwürde der

Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Ziadinov Iskenderali

aus Osch, Kirgisien

Genehmigt auf Antrag von

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Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models

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Abstract

Echinococcosis is a major emerging zoonosis in central Asia. A cross-sectional study of dogs in four villages in rural Kyrgyzstan was undertaken to investigate the epidemiology and transmission of *Echinococcus* spp. A total of 466 dogs were examined by arecoline purgation for the presence of *Echinococcus granulosus* and *Echinococcus multilocularis*. In addition, a faecal sample from each dog was examined for taeniid eggs. Any taeniid eggs found were investigated using PCR techniques (multiplex and single target PCR) to improve the diagnostic sensitivity by confirming the presence of *Echinococcus* spp. and to identify *E. granulosus* strains. A total of 83 (18%) dogs had either *E. granulosus* adults in purge material and/or *E. granulosus* eggs in their faeces as confirmed by PCR. Three genotypes of *E. granulosus*: G1, G4 and the G6/7 complex were shown to be present in these dogs through subsequent sequence analysis. Purge analysis combined with PCR identified 50 dogs that were infected with adult *E. multilocularis* and/or had *E. multilocularis* eggs in their faeces (11%). Bayesian techniques were employed to estimate the true prevalence, the diagnostic sensitivity and specificity of the procedures used and the transmission parameters. The sensitivity of arecoline purgation for the detection of echinococcosis in dogs was rather low, with a value of 38% (Credible intervals (CIs) 27-50%) for *E. granulosus* and 21% (CIs 11-34%) for *E. multilocularis*. The specificity of arecoline purgation was assumed to be 100%. The sensitivity of coproscopy followed by PCR of the isolated eggs was calculated as 78% (CIs 57-87%) for *E. granulosus* and 50% (CIs 29-72%) for *E. multilocularis* with specificity of 93% (CIs 88-96%) and 100% (CIs 97-100), respectively. The 93% specificity of the coprological-PCR for *E .granulosus* could suggest coprophagia rather than true infections. After adjusting for the sensitivity of the diagnostic procedures, the estimated true prevalence of infection of *E. granulosus* was 19% (CIs 15-25%) and the infection pressure in the dog population was estimated to be 0.29 infections per year (CIs 0.014-0.75). Logistic regression

analysis failed to identify any significant risk factors for infections for *E. granulosus*. After adjusting for the sensitivity of the test procedures, the estimated true prevalence was 18% (CIs 12-30%). Dogs that were restrained had a significantly lower prevalence of *E. multilocularis* of 11% (CIs 6-29%) compared with 26% in free-roaming dogs (CIs 17-44%) and independently within these groups hunting dogs were more likely to be infected than non-hunting dogs.

Keywords: Echinococcus granulosus; Echinococcus multilocularis; Arecoline hydrobromide; PCR; Kyrgyzstan; Epidemiology; Dog; Mathematical modelling; Bayesian analysis; Diagnostic tests

1. Introduction

Echinococcus granulosus and *Echinococcus multilocularis* are both important zoonoses. *Echinococcus granulosus* causes human cystic echinococcosis (CE). This cestode utilises dogs as definitive hosts, has a global distribution and is a problem in many areas (for example central Asia, China, South America, Northern Africa, Australia) where grazing livestock are the major intermediate hosts (Eckert and Deplazes, 2004; Jenkins et al., 2005). Different strains of *E. granulosus* exist, which, according to their preferred intermediate hosts, are called the 'sheep strain' (G1), the 'horse strain' (G4) (or *Echinococcus equinus*) or the 'pig/camel strain' (G6/7) (Thompson and McManus, 2002; Obwaller et al., 2004). In central Asia, there has been a re-emergence of CE in recent years following the collapse of the Soviet Union (Torgerson et al., 2006). In Kyrgyzstan, a mountainous country in central Asia with a population of about 5 million, the annual reported incidence of CE has increased from five to 20 cases per 100,000 person-years between 1991 and 2002 (Torgerson et al., 2003a; Torgerson et al., 2006).

Echinococcus multilocularis, which causes human alveolar echinococcosis (AE) also has a widespread distribution, but only in the northern hemisphere and is endemic in Kyrgyzstan (Shaikenov, 2006). The natural definitive hosts of *E. multilocularis* include foxes. However, dogs are also susceptible definitive hosts; high prevalences in dogs have been recorded in China (Shi, 1995; Budke et al., 2004, 2005a, b) and dog ownership has been shown to be associated with AE infection (Craig, 2006).

Arecoline purgation is known to have a low sensitivity for the diagnosis of *E. granulosus* infection (Schantz, 1997) and no attempt has been made to calculate the sensitivity of this technique for the detection of *E. multilocularis* infection. Techniques based on taeniid egg isolation and subsequent DNA analysis using PCR have been developed for the diagnosis of echinococcosis in definitive hosts (Mathis et al., 1996; Stefanic et al., 2004; Trachsel et al., 2007). These will not detect prepatent infections and therefore will have a

sensitivity of less than one. In addition there is little data on the evaluation of these tests based on samples from endemic areas. Therefore the Hui–Walter model for evaluating diagnostic tests in the absence of any absolute test (Hui and Walter, 1980; Toft et al., 2005) has been used to gain a better insight into the diagnostic performance of these tests in a highly endemic area of Kyrgyzstan.

Field data for the analysis of the transmission dynamics of *Echinococcus* spp. in dogs have been used on a number of occasions (for example see Budke et al., 2005b). However, test results have largely been used in a deterministic fashion rather than incorporating diagnostic test uncertainty into the analysis. This could give inaccurate estimates of transmission parameters such as the number infections per year. Therefore a Bayesian framework was used to incorporate diagnostic uncertainty to gain a more realistic insight into this transmission parameter.

There are few detailed studies of the prevalence and abundance of *Echinococcus* spp. in dogs in Kyrgyzstan and the factors that are associated with infection of dogs. The results of a cross-sectional study can be used to form hypotheses regarding the epidemiology and causality of risk factors for echinococcosis. In addition, the information can be used as an aid to design intervention strategies to prevent human echinococcosis. Therefore, an epidemiological study of canine echinococcosis was undertaken in Naryn Oblast in Southern Kyrgyzstan, a district with a reported hospital incidence of CE (in 2000) of more than 15 cases per 100,000 per year (Torgerson et al., 2003).

2. Materials and methods

2.1. Animals and study area

At-Bashy district has a population of about 50,000 (unpublished government figures) and is located at an altitude of 2,000 m in the south-east part of Kyrgyzstan in Naryn

province. The town of At-Bashy is the principal regional centre and has a population of about 10,000 inhabitants. There are a total of approximately 20 smaller villages in the district. Livestock husbandry is the principle occupation of the local inhabitants who are mainly ethnic Kyrgyz with small numbers of Uzbeks and Uigurs. Most dogs are kept to assist with livestock, security and hunting, and the majority are of no particular breed. A total of 466 owned dogs were studied from four settlements during August-September 2005. The total dog population in these villages is believed to be approximately 4,000-4,500 (unpublished figures from government veterinary services). A cluster sampling technique was undertaken and non-adjacent village districts were chosen to avoid, as far as possible, autocorrelation. Each district consisted of approximately 10 blocks or about 0.5 km^2. To minimise bias, every household in the district selected was invited to have their dogs participate in the study and each dog presented was sampled with the exception of approximately 14 pregnant bitches and small puppies to which arecoline could not be administered. In the town of At-Bashy itself, six districts were sampled consisting of 350 dogs. In three surrounding villages (within a distance of 15 km), five additional districts and 116 dogs were sampled. Approximately 10% of the households in the targeted population participated in the study.

2.2. Sample collection

All dogs were treated with arecoline hydrobromide (Boehringer Ingelheim) at a dose rate of 7 mg/kg in an aqueous solution of concentration 4 g/L. The required solution was administered via a dosing gun. A sample of faeces (collected immediately following administration of the arecoline but before the animal purged) was obtained from each dog and stored in 70% ethanol. Arecoline treatment often resulted in the dog defaecating immediately. This facilitated the collection of a fresh faecal sample. Otherwise, a blunt spatula was used to collect a rectal sample with the dog appropriately restrained. Purgation of the entire intestinal contents usually occurred after approximately 30 min and this material was collected and

stored separately. Any dogs that failed to purge were treated on a second occasion 1 h later. Appropriate protective clothing, including mask and gloves, was worn by all operators. The purge material was carefully analysed in a large, black, flat-bottomed basin. The material was repeatedly washed with water, parasites allowed to sediment and the supernatant removed. All observed parasites and sedimented material believed to contain any parasites were collected and preserved in 70% ethanol. In addition, any material that was not removed for examination in the laboratory was collected and incinerated. These procedures were approved by the local veterinary authorities. After purgation, the dogs' owners were given information about canine-transmitted zoonoses, in particular *Echinococcus*. All material collected was first frozen at -80^{o} C for 5 days and transported to the Institute of Parasitology, University of Zürich, for further investigation.

2.3. Diagnostic strategy.

Echinococcus granulosus and *E. multilocularis* worms were identified and differentiated microscopically from the material collected following purgation. Other parasites found in the purge material (large taeniids and ascaridoids) were also identified but the results are not reported here.

Echinococcus multilocularis or *E. granulosus* were identified by morphological characteristics under light microscopy, and differentiated on the basis of relative size and, in particular, the position of the genital pore. Based on the morphological identification, samples were assigned as purge-positive or -negative for the respective *Echinococcus* sp. Any parasites for which there was possible ambiguity were subsequently analysed by PCR (see below) to confirm the specific identification. In addition, some parasites that had been identified morphologically were also analysed by PCR (both as positive controls and in the case of *E. granulosus* to confirm the parasite genotype. A sample of 10 worms was used, or all worms if fewer were available.

Faecal samples were examined for the presence of taeniid eggs. Taeniid eggs were isolated by a combination of sequential sieving, flotation of the eggs in zinc chloride solution and egg detection using an inverted microscope (Mathis et al., 1996). Taeniid eggs isolated from faeces were also examined using PCR; those from which PCR products were obtained are termed PCR-positive for *Echinococcus* and those from which no PCR products were obtained were termed PCR-negative for *Echinococcus*. If PCR products were obtained, they were identified as *E. granulosus* or *E. multilocularis*. Dogs that were either purge-positive and/or *Echinococcus* PCR-positive were assigned as *E. granulosus* and/or *E. multilocularis* positive in the initial analysis. The investigation thus used two parallel diagnostic strategies on all dogs. The first was examination of a faecal sample collected from each dog and examined for the presence of taeniid eggs. If taeniid eggs were detected, these were examined by PCR to determine if these eggs were those of *Echinococcus* spp. A dog was assigned as negative if there were either i) no taeniid eggs detected in the faecal sample or ii) taeniid eggs were detected in the faecal sample by coproscopy but these eggs were subsequently negative for the *Echinococcus* spp.-specific PCR. The second diagnostic procedure consisted of collecting the purged material that was subsequently voided and examined directly for the presence of adult *Echinococcus* spp. with confirmation by PCR from adult worm tissue where appropriate. A dog was assigned as negative with this second test if no adult *Echinococcus* specimens could be found in the purge material and positive if at least one or more specimens were found.

2.4. DNA isolation, PCR and cloning

DNA isolation from purged worms and from taeniid eggs was as described previously (Stefanic et al., 2004). All faecal samples were analysed for the presence of taeniid eggs and positive samples were subsequently analysed by PCR. Initially, a PCR-specific probe for *E.*

granulosus G1 strain (Stefanic et al., 2004) was used on egg-DNA. During the study, a newly developed multiplex PCR for simultaneous detection of *E. granulosus* (all strains), *E. multilocularis* and *Taenia* spp. was applied to all samples (Trachsel et al., 2007) and this was the principal diagnostic test used. The results from the initial PCR were used to assist identification of the strains of *E. granulosus* detected. Negative controls (distilled water or negative sample) and a positive control (DNA extract from adult *Echinococcus*) were included in each PCR. Multiplex PCR products of *E. granulosus* (117 bp) were extracted from agarose gel using NucleoSpin Extract II (Macherey-Nagel, Germany) and PCR product cloning was performed by using TOPO TA Cloning Kits (Invitrogen, Carlsbad, CA). DNA sequencing was performed by a private company (Microsynth, Balgach, Switzerland).

2.5. Questionnaire

For all dogs examined, a questionnaire was completed with the owner during an interview. The questionnaire was in both Russian and the Kyrgyz language. The questionnaire was structured in three parts. The first included general information about the owner, name of the village and date. The second included information on the dog's sex, breed, type of dog, as well as several questions on the dog's diet and potential risk factors such as type of dog (pet, guard dog, shepherd dog or hunting dog), restraint (when not working) and if the dogs accompanied the farmers when they went to summer pastures that could influence infection status. The dog's age was estimated by questioning the owner. All questionnaires were identified by a unique numerical identification and general GPS coordinates for each village. To ensure cultural appropriateness of the questionnaire and to guarantee that each question was fully understood, the questionnaire was designed by several of the authors who have language skills in Russian, Kygyz and English, and was pre-tested in a small unpublished pilot study. The questionnaire was undertaken with each dog owner by one of the co-authors who was a native speaker of both Russian and Kyrgyz.

2.6. Analyses

For initial analysis, a dog was considered infected if adult *Echinococcus* was isolated in a purge sample or if a taeniid egg, isolated from the faeces, was PCR-positive for *Echinococcus*. The dog was considered not infected if both diagnostic procedures were negative. This then produced a series of binomial results which were analysed by logistic regression to indicate if there was any variation of infection between various risk factors associated with the dogs and the district of the village. Any significant factors were retained for further analysis. Statistical testing and logistic modelling were performed with R 2.2.0 (The R Foundation for Statistical Computing, http://CRAN.R-project.org). Due to the sampling technique used, a fixed-effects model was compared to a multi-level model with the district of sampling analysed as a random effect using the lme function of R. The optimal model description of the data was chosen based on Akaike Information Critera (AIC) which is available as a function of R. The results of the logistic regression were used to divide the dog population into sub-populations that were likely to have different prevalences.

As two diagnostic procedures were used to determine the infection status in these dogs, this provided a means for estimating the diagnostic parameters if two or more dog populations, which are likely to have different prevalences, are available for investigation using the Hui–Walter model for evaluating diagnostic tests in the absence of an absolute standard (Hui and Walter, 1980; Toft et al., 2005). In this case different populations were indicated by the logistic regression. In addition, the mathematical models previously used suggest that there is an age-dependent prevalence of infection for these parasites (Torgerson and Heath, 2003; Torgerson, 2006) and so age stratification of the data will result in populations with varying prevalences.

For further analysis of the data, it was initially assumed that the specificity of both diagnostic test procedures was 100%. Bayesian techniques were used to estimate unknown

sensitivities of the two diagnostic procedures using an appropriate spreadsheet model and macro programming in Excel (Rapsch et al., 2006). It was also assumed that the sensitivities of the tests were correlated and parameters for the model were applied accordingly. Uniform uninformative priors for the parameters were used in every case unless otherwise stated. Due to the variation in prevalence with age and possible variation in prevalence according to risk factors, it was possible to calculate the unknown sensitivities of the two tests, and any possible dependencies of the two test sensitivities, using the Hui Walter Model.

A second scenario explored was the possibility that some of the eggs identified by PCR were not from patent infections but due to coprophagia (dogs eating faeces containing *Echinococcus* eggs and which had simply passed through the intestine). This could be a source of false positives and hence decrease diagnostic specificity of the true infection status. Again all the parameters can be resolved in this model as the arecoline purgation test is fixed with a specificity of one and hence there will be no conditional dependency of the specificities (see below).

Finally, the specificity of arecoline purgation was allowed to vary on the basis that it may be possible to misidentify *E. granulosus* and *E. multilocularis* morphologically. This was the only scenario in which it was not possible to analyse conditional dependence of the two tests if all parameters were calculated simultaneously. Gibbs-sampling was used to estimate the conditional probabilities of the test parameters and the unknown true prevalence. For the correlation of test parameters, a Metropolis-Hastings routine was incorporated into the Markov Chain with the limits of the parameters conditional on the values of the other parameters (Gardner et al., 2000).

Both *E. granulosus* and *E. multilocularis* infection pressures were estimated using Bayesian techniques and in the case of prevalence models, incorporating diagnostic test uncertainty (for equations and implementation of the analysis see below). First, it was assumed there was herd immunity regulating the parasite population and comparing the model

fit by assigning the probability of developing immunity on exposure to zero. Parameters for infection pressure, rate of loss and acquisition of immunity were given bounded uniform (uninformative) prior distributions in the analysis. For *E. granulosus* a uniform non-informative prior was used for the life expectancy ($1/\mu$) of the parasite. For the life expectancy of *E. multilocularis,* due to convergence problems of a non-informative prior, the prior distribution was estimated from experimental work. Two prior distributions were analysed: a prior life expectancy of 4 months (with a S.D. of 1 month) and a prior life expectancy of 6 months (with a S.D. of 1 month). These were estimated from the results of experimental infections of dogs with *E. multilocularis* (Lukashenko, 1975; Kapel et al., 2006). Abundance models were also analysed utilising the numbers of parasites observed in each dog as a result of arecoline purgation. In this case, it was not possible to adjust parameters for diagnostic sensitivity.

2.7. *Mathematical model and Bayesian analysis*

The parameters of the transmission model are a, β, γ & μ, where a is the probability of immunity on exposure, β is the infection pressure, γ is the rate of loss of immunity and μ is the rate of loss of infection. The expected prevalence in dogs of age t is $P(t)$ and the diagnostic parameters are $Se_1, Se_2, Sp_1, Sp_2, \sigma Se$ and σSp (where Se and Sp are the sensitivity and specificity of the diagnostic tests and σSe and σSp are the correlation coefficient of the tests' sensitivities and specificities respectively). The estimates of these were based on the joint posterior probability distribution of $\psi = (a, \beta, \mu, \gamma, Se_1, Se_2, Sp_1, Sp_2, \sigma Se, \sigma Sp)$ given the data Z

$$\lambda(\psi / Z) = \frac{\lambda(Z / \psi)\lambda(\psi)}{\lambda(Z)}$$

Credible starting values for $Se_1, Se_2, \sigma Se, Sp_1, Sp_2, \sigma Sp$ and β and μ, γ and a are assigned. The expected probability of infection $P(t_i)$ in each animal i at age t is calculated. If $a \neq 0$ then $P(t_i)$

is calculated from the solution of the following equations (Roberts et al., 1986; Torgerson and Heath 2003):

$$\frac{dY(t)}{dt} = -[\gamma + \mu]Y(t) + a\beta S(t)$$

$$\frac{dX(t)}{dt} = -[\beta + \mu]X(t) + \beta[1-a]S(t) + \gamma Y(t) \qquad (1)$$

$$\frac{dS(t)}{dt} = \gamma(1 - S(t)) - \alpha\beta S(t)$$

$P(t) = X(t) + Y(t)$

$X(t)$ = proportion of dogs at age t infected but not immune, $Y(t)$ = proportion of dogs infected at age t but immune to further infection (so $P(t) = X(t)+Y(t)$ where $P(t)$ is the proportion dogs infected at age t) and S(t) is the proportion of dogs susceptible to infection at age t. The differential equations were integrated numerically to give the expected value of $P(t)$

If $a = 0$ then

$$P(t) = \frac{\beta}{\beta + \mu}[1 - \exp\{-(\mu + \beta)t\}] \qquad (2)$$

Using starting values of Se_1, Se_2, Sp_1, Sp_2 , σSe and σSp, the actual probability π_i of the ith dog being infected (I) given one of the four possible combinations of test results is calculated using:

$$\pi_i(I/+ve_1,+ve_2) = \frac{[Se_1 \times Se_2 + \sigma Se] \times P(t_i)}{[Se_1 \times Se_2 + \sigma Se] \times P(t_i) + [\ (1-Sp_1) \times (1-Sp_2) + \sigma Sp] \times (1 - P(t_i))}$$

$$\pi_i(I/+ve_1,-ve_2) = \frac{[Se_1 \times (1-Se_2) - \sigma Se] \times P(t_i)}{[Se_1 \times (1-Se_2) - \sigma Se] \times P(t_i) + [\ (1-Sp_1) \times Sp_2 - \sigma Sp] \times (1 - P(t_i))} \qquad (3)$$

$$\pi_i(I/-ve_1,+ve_2) = \frac{[(1-Se_1) \times Se_2 - \sigma Se] \times P(t_i)}{[(1-Se_1) \times Se_2 - \sigma Se] \times P(t_i) + [\ Sp_1 \times (1-Sp_2) - \sigma Sp] \times (1 - P(t_i))}$$

$$\pi_i(I/-ve_1,-ve_2) = \frac{[(1-Se_1) \times (1-Se_2) + \sigma Se] \times P(t_i)}{[(1-Se_1) \times (1-Se_2) + \sigma Se] \times P(t_i) + [Sp_1 \times Sp_2 + \sigma Sp] \times (1 - P(t_i))}$$

where the expected probability of infection for each dog i at age t is $P(t_i)$. Here, $\pi_i(I/+ve_1,+ve_2)$ denotes the probability of infection given that both tests are positive, etc. In

the case where at least one of the tests had Sp =1, π_i was always 1. Likewise when at least one of the tests had Sp = 1, σSp was always 0 as the limits of σSp (Gardner et al., 2000) are:

$$\max(-(1-Sp_1) \times (1-Sp_2), -Sp_1 \times Sp_2) \leq \sigma Sp \leq \min(Sp_1 \times (1-Sp_2), Sp_2 \times (1-Sp_1)).$$

Thus, provided that at least 1 test has Sp = 1, the model can be resolved.

A random binomial variable taking values of 1 with probability π_i and 0 with probability 1- π_i for each animal i was generated. This is the animal's updated true disease status D. Updates of Se_1, Se_2 were calculated using a Beta distributed random variable as follows. Updated Se_h (h = 1,2) is generated as a random variable from:

$$Se_h \sim \text{Beta}\ (c_h+c_p,\ d_h+d_p)$$

where c_h and d_h are the number of dogs tested positive by test h but assigned as true disease status 1 (infected) or 0 (not infected), respectively. The parameters c_p and d_p are the Beta priors which can be varied depending on any prior information on the test sensitivity. In this case both c_p and d_p = 1 as non-informative uniform distributions were used. Updated Sp_h (h = 1,2) was calculated in a similar manner. This is the Gibbs sampler which is followed by one step of a Metropolis Hastings Alogarithm to update σSe and β , μ, a and γ .

To update σSe, randomly pick a new value of σSe denoted σSe* from an appropriate proposed distribution which in this case is a random variable from a normal distribution of mean σSe and S.D. 0.1 and constrained to lie between:

$$\max\ (-(1-Se_1)(1-Se_2), -Se_1,Se_2) \leq \sigma Se^* \leq \min(Se_1(1-Se_2),Se_2(1-Se_1))$$

where Se_1 and Se_2 are the values of the two test sensitivities from the last iteration.

The probability R of accepting the new state is:

$$R = \min\left[1, \frac{\lambda(W / \sigma Se^*, \sigma Sp, Se_1, Se_2, Sp_1, Sp_2, P_{i..n})}{\lambda(W / \sigma Se, \sigma Sp, Se_1, Se_2, Sp_1, Sp_2, P_{i..n})} \times \frac{\lambda(\sigma Se^*)}{\lambda(\sigma Se)} \times \frac{q(\sigma Se)}{q(\sigma Se^*)}\right]$$

$\lambda(\sigma Se^*)$ is the probability of picking σSe* from the prior distribution. The probability of choosing the σSe* from the proposed distribution is $q(\sigma Se^*)$ whereas the probability of

proposing σSe conditional of starting at the new state σSe^* (a move not actually made) is $q(\sigma \mathrm{Se})$. The likelihood function in this case is the likelihood of the observed test result W given the values of σSe, σSp, Se_1,Se_2,Sp_1, Sp_2 and $P_{i..n}$ and is given by

$$\lambda(W / \sigma Se, \sigma Sp, Se_1, Se_2, Sp_1, Sp_2, P_{i..n}) =$$

$$\prod_{j=1}^{n_1} (Se_1 \times Se_2 + \sigma Se) \times P(t_j) + ([1 - Sp_1] \times [1 - Sp_2] + \sigma Sp) \times (1 - P(t_j))$$

$$\times \prod_{k=1}^{n_2} (Se_1 \times (1 - Se_2) - \sigma Se) \times P(t_k) + ([1 - Sp_1] \times Sp_2 - \sigma Sp) \times (1 - P(t_k)) \qquad (4)$$

$$\times \prod_{l=1}^{n_3} (Se_2 \times (1 - Se_1) - \sigma Se) \times P(t_l) + ([1 - Sp_2] \times Sp_1 - \sigma Sp) \times (1 - P(t_l))$$

$$\times \prod_{m=1}^{n_4} [(1 - Se_1) \times (1 - Se_2) + \sigma Se] \times P(t_m) + (Sp_1 \times Sp_2 + \sigma Sp) \times (1 - P(t_m))$$

Where n_1, n_2, n_3 & n_4 are the numbers testing positive to both tests, positive to test 1, positive to test 2 and negative to both tests, respectively, and where $P(t_j)$ is the probability of infection of the jth dog at age t testing positive to both tests, $P(t_k)$ is the probability of infection of the kth dog at age t testing positive to test 1, $P(t_l)$ is the probability of infection of the lth dog at age t testing positive to test 2 and $P(t_m)$ is the probability of infection of the mth dog at age t testing negative to both tests. A standard uniform random variable V is generated that is distributed between 0 and 1. If V is less than R, then the proposed new state is accepted and σSe becomes σSe*, otherwise it continues in the original state. In simulations where either Sp_1 or Sp_2 = 1, σSp = 0. Following update of σSe, the transmission parameters a, β, γ and μ, are also updated in a similar manner. The likelihood function is:

$$\lambda(D/a, \beta, \gamma, \mu) = \prod_{i=1}^{n} P(t_i)^{x_i} (1 - P(t_i))^{1 - x_i}$$

where n = number of individuals in the population; x_i = updated true infection status (1 or 0) of individual i

$P(t_i)$ = predicted probability of infection of individual i at age t and is calculated according to equation 1 or 2; D is the true infection status.

In the simulations where the prevalence was modelled according to risk factors, *P(t)* is simply replaced by *P(d)* where *P(d)* is the true prevalence of group *d*. The probability of infection given the test results of each dog *i* is then calculated according to equation (3). The likelihood function is the same (eq 4) and the Metropolis Hastings algorithm is implemented in a similar manner.

Convergence in each case was assessed visually and compared to stationary Markov chains originating from different starting values. Autocorrelation was investigated by analysing sub samples of the chain. Parameter values and their credibility intervals were estimated from 20,000 iterations of the stationary Markov Chain.

For abundance models, the equations reported in Torgerson and Heath (2003) were utilized. Here the expected number of parasites $m(t_i)$ in each dog *i* at age *t* can be modeled as:

$$m(t_i) = \frac{\alpha h^2}{(\gamma + \alpha h)(\mu - \gamma - \alpha h)}\left[\exp - \{(\gamma + \alpha h)\, t\} - \exp - \{\mu\, t\}\right] + \frac{\gamma h}{\mu\,(\gamma + \alpha h)}\left[1 - \exp - \{\mu\, t\}\right] \qquad (5)$$

where h is the infection pressure (parasites per year), α is the probability of immunity on exposure, γ is the rate of loss of immunity and μ is the per capita death rate of the parasite. When there is no parasite-induced host immunity (i.e. $\alpha = 0$) this is equivalent to:

$$m(t_i) = \frac{h}{\mu}\left[1 - \exp - \{\mu\, t\}\right] \qquad (6)$$

where h is the infection pressure in terms of parasites per year, μ is the rate of parasite loss ($1/\mu$ is the mean survival time of the parasite), α is a parameter influencing the rate at which immunity is acquired, and γ is the loss of immunity. For the Bayesian analysis, the negative binomial likelihood function was used:

$$\prod_{i=1}^{n} \frac{\Gamma(k + s_i)}{\Gamma(k)\, s_i!}\left(\frac{m(t_i)}{k + m(t_i)}\right)^{s_i}\left(\frac{k}{k + m(t_i)}\right)^{k}$$

where s_i is the number of parasites observed in the *i*th dog, k is the negative binomial constant and Γ represents the gamma distribution. Reasonable starting values were assigned for parameters h, μ, α, γ and k. Expected values of $m(t_i)$ were calculated for each dog using

equations (5) or (6). To update *h*, randomly pick a new value of *h,* denoted *h**, from an appropriate proposed function. The probability *R* of accepting the new state is:

$$R = \min\left[1, \frac{\lambda(M/h^*,\mu,\gamma,\alpha,k)}{\lambda(M/h,\mu,\gamma,\alpha,k)} \times \frac{\lambda(h^*)}{\lambda(h)} \times \frac{q(h)}{q(h^*)}\right]$$

$\lambda(h')$ is the probability of picking h' from the prior distribution and $\lambda(M/h,\mu,\gamma,\alpha,k)$ is the likelihood of the data given the present set of parameters. A standard uniform random variable *V* is generated that is distributed between 0 and 1. If *V* is less than *R*, then the proposed new state is accepted and *h* becomes *h**, otherwise it continues in the original state. The other parameters are updated in an identical manner.

3. Results

3.1. Purgation, PCR and sequencing

A total of 466 dogs were sampled. Of these, 34 samples were positive on purgation for *E. granulosus*. Microscopic examination of the faecal samples revealed taeniid eggs in 19 of these samples, and all were identified as the G1 strain of *E. granulosus* using PCR. The other 15 samples were negative for eggs on microscopic examination. Microscopic examination followed by egg-PCRs detected a total of 68 dogs with *E. granulosus* eggs in the faeces. Thus the total number of dogs that were either *E. granulosus* purge-positive and/or *E. granulosus* egg-positive following microscopic examination was 83 (17%) (Table 1).

PCR specific for the G1 strain of *E. granulosus* was positive on DNA from eggs or tissue from worms in 60 dogs. The *E. granulosus*-specific amplicons of the multiplex PCR of the remaining 22 samples were further analysed by sequencing, revealing highest sequence homology (> 99%) with the G6/7 strain sequence (accession numbers **AY462128**, **AY462126** and **L49456**) in seven of these samples. Correspondingly, eight samples were identified as the G1 strain (highest identities with GenBank entries **AY462129**, **AF297617** and **AB031350**).

One sample showed identity with the *E. granulosus* G4 strain (GenBank entries **AF346403**), and no sequence information could be obtained for six samples. Therefore, of the 83 samples that were confirmed to be positive by PCRs on eggs isolated from the dogs' faeces or tissue from the purged worms, 69 were confirmed as the G1strain, seven as the G6/G7strain, and one as the G4 strain; six samples were not identified.

For *E. multilocularis*, 17 samples (3.6%) were purge-positive. A total of 42 samples were positive by microscopic faecal examination followed by multiplex PCR on DNA from isolated taeniid eggs. Eight samples were purge-positive but had no eggs in the faeces. All eight samples proved to be *E. multilocularis* by PCR on material isolated directly from the purged worms. Thus, a total of 50 dogs were proven to be infected with *E. multilocularis* (Table 1). Faecal examination and purge results demonstrated that six dogs were co-infected with *E. granulosus* and *E. multilocularis*. Of these, two were co-infected with the G6/7 strain of *E. granulosus* and *E. multilocularis* and four were co-infected with the G1 strain of *E. granulosus* and *E. multilocularis*.

A further two samples were purge-positive for *E. granulosus* and were negative on microscopic examination of the faeces. However, PCR on adult worm material indicated mixed infections with *E. granulosus* and *E. multilocularis*.

3.2. Risk factor analysis

Multivariate logistic analysis suggested that dogs that were restrained all the time were less likely to be infected with *E. multilocularis* compared with dogs that were allowed to roam some or all the time, and that dogs used for hunting had a higher probability of infection compared with those not used for hunting ($P < 0.05$). No risk factors were significant for infection with *E. granulosus*. (Table 2). The multi-level model with random effect of district of sampling had a marginally higher AIC with hunting dogs and freely roaming dogs

significantly different. Thus the fixed effects model was adopted as the best description of the data with no evidence of clustering of infection.

3.3. Modelling

The purgation and egg isolation followed by PCR gave a series of results (summarised in Table 1) but not necessarily the true infection status, particularly when tests were negative. The Hui–Walter model was used to estimate diagnostic parameters and the true prevalence of infection given the series of test results. This suggested that the sensitivity of arecoline purgation for detecting infection of dogs with *E. granulosus* was approximately 38% (95% Credible Intervals (CIs) 27-50%) (Table 3). With *E. multilocularis,* the sensitivity for detecting the parasite was 21% (CIs 11-34%). The sieving technique followed by PCR of the isolated taeniid eggs had a sensitivity of 78% (CIs 57-87%) for *E. granulosus* detection. Similarly, for *E. multilocularis* the sensitivity was 50% (29-72%). The specificity of the *E. granulosus* PCR was 93% (CIs 88-96%) and that of *E. multilocularis* 100% (97-100%). The specificity of arecoline converged on one indicating little or no morphological misidentification of the two *Echinococcus* spp. The median value of the correlation coefficient for the sensitivity of arecoline purgation and egg isolation techniques was -0.02 (CIs -0.09-0.12). For the detection of *E. multilocularis,* these were 0.019 (-0.091-0.065). The adjusted prevalence of *E. granulosus* was 19% (15-25%), whilst that of *E. multilocularis* was 18% (12-30%). Free roaming dogs had a higher adjusted prevalence of *E. multilocularis* of approximately 26% (17-44%) compared with that of 11% (6-29%) in restrained dogs ($P < 0.05$). Within these two groups, hunting dogs had significantly higher prevalences (Table 3). For both *E. granulosus* and *E. multilocularis*, the probability of developing immunity was not significantly different from zero. Therefore the simpler model with these parameters set to zero was used to calculate the transmission parameters. The variation of the estimated true prevalences with age, adjusted for diagnostic uncertainty, is displayed in Figs. 1 and 2. The

dogs were exposed to a median of 0.29 (0.014-0.82) infections per year of *E. granulosus* (Table 4). The unadjusted infection pressure *h* was estimated at 59 (24.7-201.7) parasites per year for *E. granulosus.* The rate of loss of *E. granulosus* converged on a median of 1.3, which gives a life expectancy of approximately 9 months for an infection using a non-informative prior. With *E. multilocularis*, the dogs that were allowed to roam had a significantly higher infection pressure of 1.29 (0.44-2.95) infections per year compared with 0.48 (0.12-1.27) ($P < 0.05$) when the life expectancy was given a prior of 4 months (μ=3). This decreased to a median of 0.42 (0.11-1.38) and 1.13 (0.39-2.78) for restrained and free roaming dogs, respectively (not significant), when the prior life expectancy was increased to 6 months (μ=2). There was no satisfactory convergence when a non-informative prior for the life expectancy was used. With *E. multilocularis*, the model converged on a median of 3.67 given the prior of 3 and the data or 3.12 given the prior and the data. This suggests a life expectancy of an infection of 3.3 months or 3.8 months given the data and prior assumptions, respectively. For *E. multilocularis*, the unadjusted infection pressure in parasites per year was estimated at 137 (28-3,327) parasites for restrained dogs and 232 (57-7,103) parasites for roaming dogs with a prior life expectancy of 4 months (μ=3). This decreased to 94 (20-2,274) and 173 (35-4,753) parasites per year, respectively, with a prior life expectancy of 6 months (μ=2). No meaningful values of the transmission parameters for hunting dogs compared with non-hunting dogs for the restrained and free roaming dogs could be calculated due to the small numbers of hunting dogs in the data set.

Sub-sampling of the Markov chains indicated that autocorrelation was not significant in calculating the Bayesian posterior distributions (data not shown).

4. Discussion

This report represents a detailed analysis the epidemiology of infection, the transmission dynamics and the diagnosis of *Echinococcus* spp. in a population of dogs from a

highly endemic area for echinococcosis. This study confirms the presence of the G1 and G4 strains together with the G6/7 strain/cluster in central Kyrgyzstan. Bart et al. (2006) have also described the G6/7 strain/cluster of *E. granulosus* from dogs in neighbouring Xinjiang province of Western China. As domestic pigs (*Sus scrofa*) are not kept in this area, dogs may be infected from local wild pigs. Alternatively, Varcasia et al. (2007) have suggested that goats are suitable hosts for the G6/7 strain/cluster and hence may be the intermediate host in Kyrgystan for this parasite.

Dogs that were co-infected with *E. multilocularis* and *E. granulosus* were detected as reported in previous studies (Stefanic et al., 2004; Xiao et al., 2006). In these reports, co-infections with *E. multilocularis* and two different strains of *E. granulosus* were detected. With the exception of the Middle East (Sadjada, 2006), there are few previous reports of the G4 strain in Asia.

Arecoline is known to provide a relatively low sensitivity for the diagnosis of *E. granulosus*. In this study, the sensitivity for *E. granulosus* appeared to be even lower than that reported by Schantz (1997) (66%); the Bayesian analysis suggested that the sensitivity was only about 38%. The sensitivity for arecoline purgation for *E. multilocularis* diagnosis is poor with approximately 21% of infected dogs detected. Arecoline purgation has only rarely been used to investigate *E. multilocularis* infections in dogs (Budke et al., 2005b) and hence there are very few data on the diagnostic performance of this technique. The level of sensitivity reported here gives it little utility for defining prevalence data unless combined with other tests. However, it is not known how useful it is at detecting heavily infected dogs which are more likely to be dangerous for transmission to humans. Lightly infected dogs are more likely to be missed either due to incomplete purgation or due to errors in examining the purge material. *Echinococcus multilocularis* is known to be able to penetrate deeply between the intestinal villi and into the crypts of Lieberkühn (Thompson and Eckert, 1983). This, combined with the parasite's smaller size, may make it more difficult to dislodge when an

animal is treated with arecoline. However, the sensitivity of arecoline was calculated by indirect mathematical techniques and necropsy studies might provide better information. The specificity of arecoline purgation converged on one, which indicates indirectly that morphological identification with PCR was accurate. Two samples which had no taeniid eggs in the faeces and had parasites that were identified morphologically as *E. granulosus* were also PCR-positive for *E. multilocularis* when the adult worms were examined, suggesting a mixed infection. In the analysis, these two samples were treated as negative for *E. multilocularis* based on purge samples as adult worms could not be identified in the purge. Inclusion of these data in the risk analysis (Table 2) does not alter the association with *E. multilocularis* infection, but does slightly increase the *P* value and odds ratio of one of the two significant variables.

The latent class model suggested that the sensitivity of microscopic faecal examination (egg isolation) followed by PCR of the eggs was 78% for *E. granulosus* and 50% for *E. multilocularis*. This technique can only detect patent infections and thus there is a limit to the sensitivity that can be achieved. It is possible that repeated faecal examinations could improve the sensitivity as has been shown elsewhere (eg fasciolosis in cattle, Rapsch et al., 2006). Some of the animals which had worms in the purge sample but had no eggs in faeces were probably pre-patent infections on the basis of morphological analysis of the recovered worms. *Echinococcus granulosus* has a prepatent period of approximately 6 weeks (Gemmell et al., 1986) and a life expectancy of approximately 10 months to 1 year (Aminzhanov, 1975). Therefore, it is unlikely that the sensitivity could be higher than 90% as this represents the proportion of the parasite's life span when it is in the mature or patent phase. Consequently, it could be argued that the sensitivity for detecting patent infections was in the order of 87% (the test is only capable of detecting patent infections, thus the proportion detected, 78% divided by the maximum detectable, 90% gives the sensitivity for patent infections). The same arguments apply for *E. multilocularis*. The prepatent period is about 28 days with the

egg production of patent infections virtually eliminated by 90 days after infection (Kapel et al., 2006) putting a limit to the detection of this parasite at about 69%. Therefore, in this study this technique detected at least 72% of patent infections.

Dogs are known to be coprophagic and it is possible that some animals consumed faeces from infected dogs which then passed through the intestine and were subsequently detected by the PCR technique. Indeed, Staebler et al. (2006) speculated that some of the rare cases of canine alveolar echinococcosis are the result of dogs consuming fox faeces. When the specificity of PCR was allowed to vary, it suggested that the specificity of the techniques was approximately 93% for the detection of *E. granulosus* but remained at close to 100% for *E. multilocularis*. This could suggest that a small number of the PCR-positive animals are due to the intestinal passage of *E. granulosus* eggs rather than true infections. Other sources of contamination such as in the laboratory were thought to be unlikely as the negative controls showed no sign of cross-contamination. However, if dogs are consuming *E. multilocularis* eggs, these eggs do not passage in sufficient quantities to be detected by the PCR test.

The detected prevalences of infection for *E. granulosus* and *E. multilocuolaris* were 18% and 11%, respectively (Table 1). To determine the test parameters and the true prevalence the Hui-Walter paradigm was exploited (Hui and Walter, 1980; Toft et al., 2005). This analysis resulted in an estimated true prevalence of 19% of *E. granulosus* and 18% for *E. multilocularis*, respectively. This upward shift in the prevalence was because some animals that were both negative on purgation and PCR-negative were nevertheless infected due to imperfect sensitivity of the two tests. A combination of arecoline purgation and egg isolation followed by PCR identified 83 infected dogs (17.8%), which gives a combined sensitivity using both techniques of approximately 87% for *E. granulosus*. Likewise, the combined sensitivity for *E. multilocularis* detection was 60%. The adjusted prevalence of 19% for *E. granulosus* compares with 28% in neighbouring Uzbekistan, 23% in Kazakhstan (farm dogs) and 15% in Tajikistan (Torgerson et al., 2006). However, the Uzbekistan and Kazakhstan

studies were reported from the results of purgation studies and are likely to substantially underestimate the true prevalence in these populations. The Tajikistan study was a necropsy study.

The adjusted prevalence of 18% of *E. multilocularis* was comparable with the estimated true prevalence (after adjusting for diagnostic sensitivity) of 19.7% recorded in China (Budke et al., 2005a). The intensity and abundance of infection were rather low for *E. granulosus* compared with previous studies. However, the abundance and intensity of infection with *E. multilocularis* was comparable with that found in China. Close contact with dogs is an obvious risk factor for human CE and has been reported as a risk factor for human AE (Yang et al., 2006). To what extent these dogs pose a risk to the local human inhabitants is presently unknown, but very high human prevalences in China have been reported in areas where the local dog population has had similar levels of infection to the present report (Budke et al., 2004).

Dogs that are allowed to roam, at least some of the time, were more likely to be infected with *E. multilocularis.* Dogs used for hunting rather than other purposes also had an independent increased risk of infection. This is consistent with possibly two sources of infection. When dogs are taken hunting they can prey on small rodents whilst dogs that are allowed to roam by their owners can also scavenge rodents. The findings in the present report support those of Budke et al. (2005a) who also found that roaming dogs were more likely to be infected on the Tibetan plateau. It is possible that some dogs with higher risks of infection (such as shepherd dogs for example, Torgerson et al., 2003b) were underrepresented in the sample. However only *E. multiloculasris* prevalences were found to vary between different types of dogs and thus it can be argued that if substantial numbers of hunting dogs are absent a higher overall prevalence of *E. multilocularis* might be expected.

This manuscript also reports the use of the Markov Chain Monte-Carlo to fit data to an established model. In addition, diagnostic uncertainty is incorporated into the analysis. This

technique gives a probability distribution for the true value of β given the data and diagnostic uncertainty. For *E. granulosus,* the model suggested that the median value of β was approximately 0.29 infectious insults a year and analysis of the rate of establishment of the infections and the variation of the measured worm burden per dog suggests that there was little evidence of parasite-induced host immunity in this population of dogs. This may be because of the relatively low infection pressure with few parasites: the mean number of parasites per dog was just 50 which is far lower than in regions such as Kazakhstan (farm dogs) or Tunisia where there is more evidence of parasite-induced host immunity. The unadjusted infection pressure was just 59 parasites per year. In this case it may be that the infection pressure was too low to stimulate immunity (Torgerson, 2006). However, because of the low sensitivity of arecoline, the true abundance is likely to be considerably higher. The detected abundance of 50 parasites per dog was lower than the 80 parasites per dog found in China by Budke et al. (2005b) using the same technique. More importantly, the detected *h* was only 59 parasites per year, which was far lower than the Chinese study (560 parasites per year) where there was evidence of a lower abundance in the older dogs suggesting herd immunity.

Because of the significantly different prevalences of *E. multilocularis* in the restrained dogs compared with the freely roaming dogs, separate values for the infection pressure for these two populations were calculated. This presents the deterministic modelling approach based on risk factors of the host population. A similar approach can also be used for populations of dogs that are separated geographically to give a spatially deterministic approach to these problems. Combined with the incorporation of diagnostic uncertainty, it represents an advance in the modelling approach to the transmission of *Echinococcus* in the definitive host. The interpretation of the quantitive data in the presence of low arecoline sensitivity is a challenge. Certainly the exposure rate of the dogs to *E. multilocularis* is comparable with that of the study in China (Budke et al., 2005b) which used the same

technique. However, it is clear that detailed necropsy studies are required to determine the utility of arecoline for quantitative data. A certain amount of caution is required when interpreting the transmission modelling, particularly when it comes to possible seasonal effects as these dogs were sampled in late summer. The long parasite life expectancy of close to 1 year will mitigate these problems for *E. granulosus*, but the short life expectancy of *E. multilocularis* means that the season of sample collection could have a substantial effect. The infection pressures reported for *E. multilocularis* assume that infection pressure is constant throughout the year which is unlikely. In Switzerland for example, differences in prevalences have been suggested between foxes sampled in the winter and the summer (Hofer et al., 2000; Stieger et al., 2002; Hegglin et al., 2007). Studies are being undertaken to resolve the issues of seasonal transmission in Kyrgyzstan.

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Legends for figures

Fig. 1. Age-stratified true prevalence of dogs infected with *Echinococcus multilocularis*.

Fig. 2. Age-stratified true prevalence of dogs infected with *Echinococcus granulosus.*

Table 1. Purgation and coproscopy followed by PCR* results of 466 dogs in Kyrgyzstan, 2005.

Parasite	Purgation and identification of adults			Microscopic faecal examination of eggs followed by PCR confirmation		Total positive	
	Abundance (95% bootstrap confidence intervals)	Number positive	%	Number positive	%	Number positive	%
E. granulosus	50 (16-97)	34	7	68	14.6	83**	18
E. multilocularis	65 (22-123)	17	4	42	9.0	50	11
Co infection with both parasites		2	0.4	5	1.1	6	1

* Multiplex PCR with DNA from isolated taeniid eggs.

**69 strain G1, 7 strain G6/G7, 1 strain G4, 6 sequence not identified, but positive on multiplex PCR as *E. granulosus*

Table 2. Multivariate analysis for possible variables associated with acquisition of canine *Echinococcus* spp. infection (n=466)

Variable	Odds ratio	Confidence Interval	P value
Dogs is tied all the time	0.39	0.199- 0.749	0.0056[#]*
Hunting dog or other type (dichotomous)	4.28	1.89-9.68	0.0005*

*Significant variables

[#]Two additional dogs may have been infected with *E. multilocularis*. (When included in the analysis the odds ratio increases to 0.4255 and p value was increased to 0.009)

Table 3. Diagnostic parameters of arecoline and PCR and estimated true prevalence of *Echinococcus* spp. in dogs (95% credible intervals) calculated from the Hui-Walter model .

	Arecoline	PCR		True prevalence		
	Sensitivity for intestinal stages	Sensitivity for eggs in faeces	Specificity	Total	Restrained dogs Hunting type, Non hunting type	Free roaming dogs Hunting type, Non hunting type
E. granulosus	38% (27-50)	78% (57-87)	93% (88-96)	19%(15-25)		
E. multilocularis	21% (11-34%)	50% (29-72)	100% (97-100)	18% (12%-30%)	11% (6-29%) 30%(14-56%), 9.4% (5-19%)	26% (17-44%) 44% (25-69), 24% (16-42%)

Table 4 Transmission parameters for *Echinococcus* spp. in dogs

	β (infections per year)		h (parasites per year)		μ (per year)	Negative binomial constant k
	Restrained dogs	Roaming dogs	Restrained dogs	Roaming dogs		
E. multilocularis	0.48 (0.12-1.27)[1]	1.29 (0.44-2.95)[1]	137(28-3327)[1]	232 (52-7103)[1]	3.67 (1.42-6.17) [1]	0.0038 (0.002-0.006)
	0.42 (0.11-1.38)[2]	1.13 (0.39-2.78)[2]	94(20-2274)[2]	173 (35-4753)[2]	3.12 (1.25-5.43)[2]	
E. granulosus[2]	0.29 (0.014-0.82)		59.0 (24.7-201.7)		1.3 (0.1-3.50)	0.0082 (0.006-0.012)

[1] A prior of mean 3, standard deviation 0.5 from a normal distribution (parasite life expectancy of 4 months) for the parameter μ

[2] A prior of mean 2, standard deviation 1 from a normal distribution (parasite life expectancy of 6 months) for the parameter μ

[3] All parameters were given uniform non informative priors

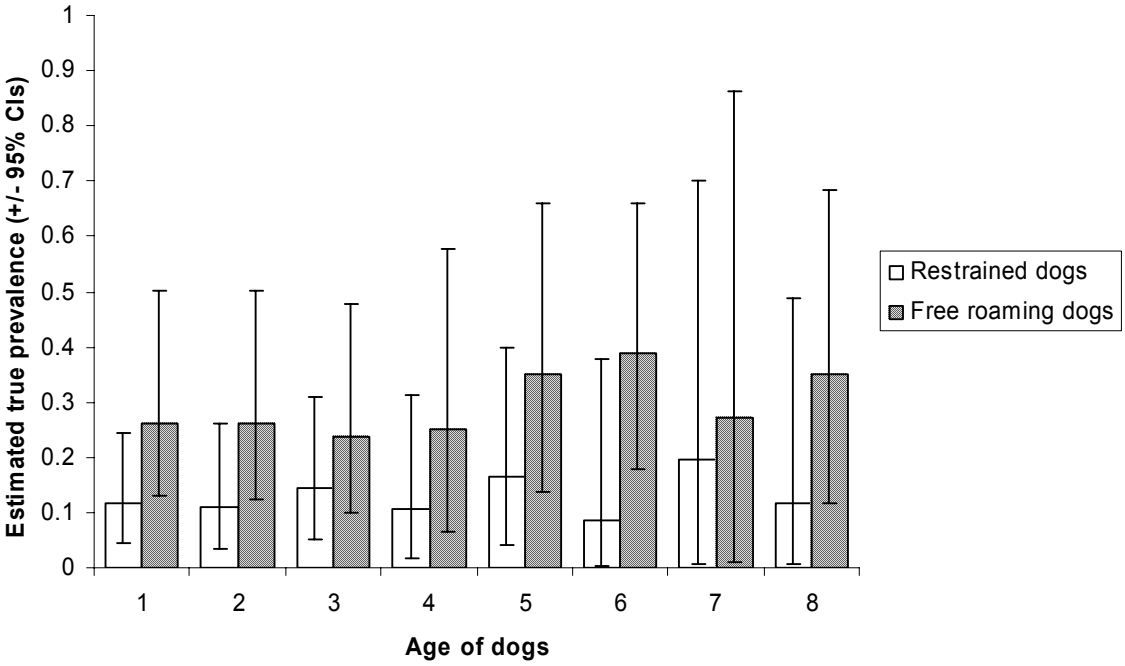
Estimated true prevalence (+/- 95% CIs)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
Age of dogs
Restrained dogs
Free roaming dogs

Figure 1.

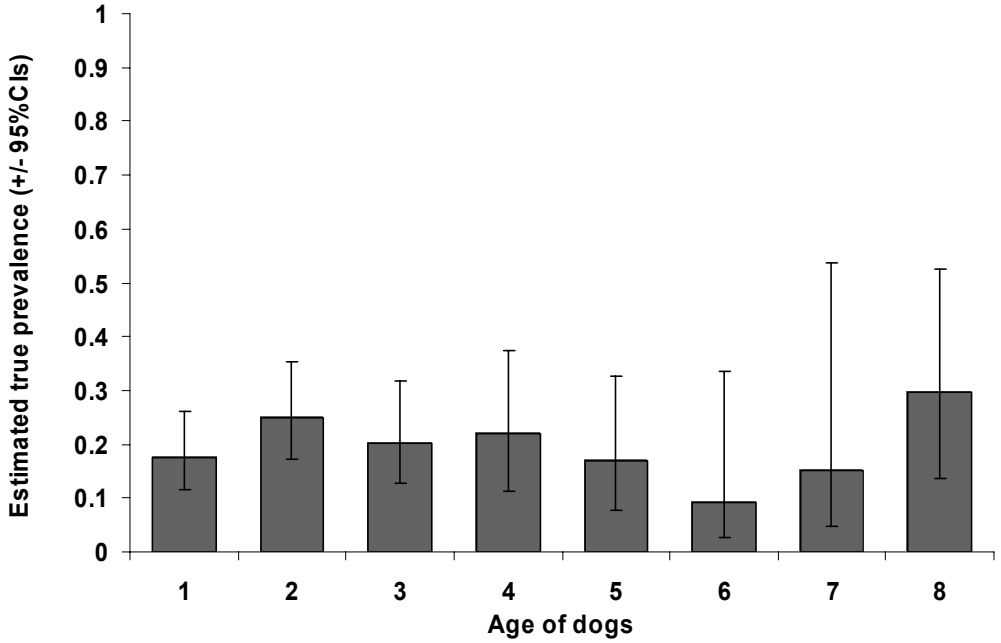
Estimated true prevalence (+/- 95%CIs)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
Age of dogs

Figure 2.

Curriculum vitae

Name	Ziadinov Iskenderali
Date of Birth	03.01.1974
Place of Birth	Bel, Osh oblast, Kyrgyzstan
Nationality	kyrgyz
State	Kyrgyzstan

Education and other qualifications

1981 – 1991	Secondary school after name N. Krupskaya in Eski-Nookat, Kyrgyzstan, 1981-1991
1993-1998	Kyrgyz agrar academy, Faculty of Veterinary Medicine, Bishkek, Kyrgyzstan,
1998	State exams in the Faculty of Veterinary Medicine, Bishkek, Kyrgyzstan
2002-2003	Member of staff researcher in the department of biotechnology and virology, Kyrgyz Research Institute of Livestock, Veterinary and Pastures, Bishkek, Kyrgyzstan
2003-2005	Scholarship ESKAS programme in the Institute of Virology University of Zurich, Switzerland
2005-2008	Doctorand in the Institute of Parasitology Vetsuisse Fakultät, Universität Zürich Winterthurerstrasse 266a 8057 Zürich

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